--90. The method of Claim 17 further comprising the additional step of:

(c) inducing cells proliferated in step (b) to differentiate by plating said cells on a fixed substrate.

--91. The method of Claim 17 further comprising the additional step of:

(c) inducing cells proliferated in step (b) to differentiate in suspension by allowing the cells to form clusters of cells without reinitiating proliferation.

--92. The method of Claim 17 wherein the source of said mammalian brain tissue is a juvenile or adult mammal.

--93. The method of Claim 17 wherein said dissociated multipotent stem cell is proliferated at least 21 days *in vitro* with substantially no differentiation.--

REMARKS

Amendment to the claims

The claims have been amended to more clearly define the present invention and to overcome the objections to the claims under § 112, as discussed in detail below. In particular, claim 17 is amended to clarify that the progenitor cells proliferated by the methods of the present invention are multipotent stem cells and are capable of self-renewal. Support for this language is found in the

specification on page 19, lines 13-16. As described in detail below, the cells proliferated by the methods of the present invention are distinguishable from other progenitor cell types that are committed to undergo terminal differentiation.

New claims 85-93 are added to further define the present invention. Support for these claims is found in the specification. These claims are discussed in further detail in the appropriate sections below.

Rejections under § 112

The specification stands objected to under 35 U.S.C. §112, first paragraph for failing how to disclose methods of culturing progenitor cells derived from tissue other than brain tissue. Claim 17 has been amended to recite that the progenitor cells are obtained from neural tissue rather than from "brain or striatal tissue" as suggested by the Examiner. In the attached declaration from Brent A. Reynolds ("the Reynolds Declaration") it is shown that stem cells obtained from neural tissue from non-brain regions (for example the conus medularis) can be proliferated and differentiated using the methods of the present invention (see Part 4, Section III of the declaration).

The Examiner also objects to the specification for failing to teach how to culture cells derived from mammalian species other than mice. In the Reynolds Declaration it is shown that stem cells obtained from both human and monkey proliferated and differentiated *in vitro* using substantially the same methods that are

disclosed in the subject application. Cells were obtained from the conus medularis of an adult Rhesus and dissociated using the same techniques set forth in Example 1 of the specification. The cells were then proliferated using substantially the same procedure as set forth in Example 5, and differentiated using substantially the same procedure as set forth in Example 6 except that the substrate used was laminin-coated glass coverslips rather than poly-L-ornithine-coated. However, the ability to use alternate substrates for cell adhesion is well-recognized by those of ordinary skill in the art.

In addition to monkey cells, cells were obtained from embryonic human neural tissue and proliferated and differentiated using substantially the same techniques as set forth in the specification. This is detailed in Part 4, Section I.(B) of the Reynolds Declaration. Thus it has been demonstrated that the disclosure of the subject application enables one skilled in the art to, without undue experimentation, proliferate and differentiate progenitor cells obtained from mammalian species such as monkeys and humans. Given the diversity of the species (from mice to man) from which it has been shown that progenitor cells can be proliferated and differentiated using the methods of the present invention, applicants have amply supported their claims to a method of proliferating a multipotent progenitor cell (i.e. stem cell) from neural tissue in any mammalian species. Accordingly, it is submitted that the rejection to the specification should be withdrawn.

The Examiner objects to the phrase in claim 17 "isolating a cell from a mammal". The Examiner states that the language does not reflect "the steps actually conducted by Applicants since Applicants first cloned out the cells." It is unclear what the Examiner means by this statement because in order to clone out cells, they are first isolated. In all events, as can be seen from Example 5 of the specification, it is not necessary to isolate a single progenitor cell and clone it in order to practice the present invention. Thus, claim 17 has been amended to more accurately reflect the scope of the present invention by replacing the phrase objected to by the Examiner with "dissociating mammalian neural tissue containing at least one multipotent stem cell". Support for this language is found on page 21, lines 10-26 of the specification, and in Example 1.

The Examiner further objects to claim 17, requiring that it be limited to "the use of mouse EGF, use of EGF at 20 ng/ml, and a particular medium". Such a limitation is overly restrictive. First the claims should not be restricted to any particular growth factor. Applicants have disclosed a number of potentially suitable growth factors. See, e.g., p. 23, lines 13-24. Applicants have also exemplified the claimed method using the two most preferable growth factors, EGF and TGFα. See, p. 16, lines 4-15; p. 23, line 26; p. 39, line 23 - p. 40, line 18. Further, applicants have provided detailed methodology for testing any of the disclosed growth factors to determine if it is suitable for achieving proliferating the cells according to the claimed method using one of the disclosed growth factors, EGF.

Thus, following the teachings of applicants' specification, one of ordinary skill in the art can, without undue experimentation, determine whether a particular growth factor is suitable for achieving proliferation according to applicants' claims. The Federal Circuit Court of Appeals has held that the necessity of one of ordinary skill in the art to engage in some experimentation to practice a claimed invention does not render the disclosure non-enabling as long as the experimentation required is not "undue." *In re Wands*, 8 USPQ2d 1400 (1988). In *In re Wands*, the fact that not all monoclonal antibody cell fusions resulted in a desired end-product and that some screening was required in order to obtain monoclonal antibodies that fit within the claim language did not constitute undue experimentation. In reaching this conclusion, the court found that the applicant's disclosure provided considerable direction and guidance on how to practice the invention and presented working examples. Similarly, the applicants of the present invention have provided working examples with detailed methodology for testing growth factors. The fact that not all growth factors will effect the proliferation of cells, and that some experimentation is required in order to ascertain which growth factors other than those specifically enabled will result in proliferation does not constitute undue experimentation. Thus the enablement requirement has been satisfied.

As to the use of mouse EGF at 20 ng/ml, it is maintained that such a limitation is overly restrictive and not reflective of the scope of protection the present invention deserves. The Examiner

suggests that in order to comply with §112 the scope of the claims must be limited to the conditions set forth in the working examples. However, as set forth above, the recognized standard is that there be sufficient disclosure for one of ordinary skill in the art to practice the claimed invention without undue experimentation. It would not be undue experimentation for the skilled lab technician to perform a titration experiment to ascertain the effects of varying concentrations of EGF on the proliferation of multipotent stem cells. In all events, the Reynolds Declaration, at Part 4, Section II, indicates that murine embryonic EGF-responsive cells were proliferated and passaged in culture medium containing 1, 10, 50, and 100 ng/ml EGF. In all cases, the rate of proliferation, number of cells generated, and morphological characteristics of the cells was identical to that seen when 20 ng/ml was used. When the cells were differentiated, neurons, astrocytes and oligodendrocytes were identified immunocytochemically. Thus the results were the same as when 20 ng/ml EGF was used, namely that multipotent stem cells were capable of proliferation and subsequent differentiation to neurons and glia.

The Examiner also requires that the claims be limited to a particular culture medium containing mouse EGF. However, the claims should not be restricted to a growth factor from a particular species (e.g., murine EGF). One of ordinary skill in the art would know to use the appropriate species-specific growth factor. In addition, one of ordinary skill in the art would be able to determine by routine experimentation which growth factors exhibit cross reactivity in other species. For example, in Part 4, Section I(A) of the Reynolds Declaration, it is shown that human recombinant EGF

was effective in inducing the proliferation of progenitor cells obtained from both monkey and humans.

The Examiner also requires that the claims be narrowed to the particular culture medium used in the working examples. Various culture media are well known in the art. To limit the scope of the patent to an exemplified culture medium would be an unwarranted limitation on the scope of protection that the invention deserves in light of the fact that altering the components of culture media is well within the ken of one of ordinary skill in the art and does not entail undue experimentation. In addition, the Applicants have disclosed several suitable media in the specification (p. 21, line 25 - p. 22, line 9) and thus should not be limited to one exemplified culture medium. Accordingly the enablement requirements, as set forth in *In re Wands*, *supra*, have been satisfied.

The Examiner requires that the claims be limited to the type of culture substrate shown to be effective in culturing the cells. However the examples in the specification demonstrate that there is no one particular substrate necessary for the proliferation and subsequent differentiation of these cells. In Example 3, dissociated cells are proliferated in a standard 96-well tissue culture plate having no particular coating. In Example 4, the cells are plated onto poly-L-ornithine-coated glass coverslips. In Example 5, dissociated cells were seeded in a T25 culture flask, lacking any substrate to which the cells could adhere. These cells proliferated in suspension, a preferred, but not necessary method of proliferation. Claim 89 sets forth this preferred method of proliferation. Support

for this language can also be found in the specification on page 25, lines 23-28. Newly added claims 90 and 91 set forth the two methods by which the proliferated progenitor cells can be induced to differentiate, either on a fixed substrate (claim 90) or in suspension (claim 91). Support for these new claims can be found on p. 26, line 25 - p. 27, line 5.

In view of the amendments to the claims and for the above reasons, it is believed that the objection to the specification under 35 U.S.C. § 112, first paragraph, is overcome. For the same reasons, it is believed that the rejection to claim 17 under § 112, first paragraph, is also overcome.

The Examiner has required that new pictures be submitted to support the claims. Formal drawings are submitted herewith.

Claims 17, 18, and 20 stand rejected under 35 U.S.C. § 112, second paragraph. Claim 17 has been amended so that the final step results in the proliferated cells and thus correlates with the preamble. New claims 90 and 91 clearly set forth that once cells have been proliferated, they can be subjected to further conditions that induce the cells to differentiate. Accordingly it is believed that the objection to claims 17, 18, and 20 is overcome.

Claims 18 and 20 stand rejected under 35 U.S.C. § 112, first paragraph. It is believed that the arguments presented above along with the data submitted in the Reynolds Declaration are sufficient in overcoming the rejection to claim 17, from which claims 18 and 20

depend. Accordingly, for the above reasons, and in light of the amendments to claims 17, 18, and 20, it is believed that the rejection to these claims is overcome.

Rejections under § 102

Claim 17 stands rejected under 35 U.S.C. § 102(a) as being anticipated by Cattaneo et al. For a proper rejection under § 102, each and every element of the claimed invention must be disclosed in the prior art reference. Claim 17 has been amended to clarify that the cells being proliferated by the methods of the present invention are multipotent stem cells. The hallmark of a multipotent stem cell is that it is capable of proliferation without limit. The cell undergoes asymmetric division to produce a daughter multipotent stem cell and a daughter cell committed to undergo terminal differentiation. The applicants were the first in their field to demonstrate methods for the proliferation and differentiation of a multipotent stem cell isolated from neural tissue, that is capable of proliferation without limit *in vitro*, and capable of differentiating into neurons and glia. Cattaneo et al. do not disclose the proliferation of a multipotent stem cell, as set forth in claim 17. The cell described by Cattaneo et al. appears to be a unipotent progenitor cell, capable of differentiation only into neurons.

In addition to not disclosing every element of claim 17, Cattaneo et al. also fail to demonstrate or suggest additional elements of the present invention that are set forth in some of the newly added dependent claims. For example, Cattaneo et al. do not

describe or suggest a method for achieving proliferation without limit (i.e., in the absence of differentiation) as set forth in claim 88. Cattaneo et al. at best demonstrate proliferation for a period of 9 days *in vitro*. However, Figure 2d, p. 763 of Cattaneo et al. indicates that the cells were also differentiating during that period, as illustrated by the increase in neurofilament staining cells. The cells proliferated by the methods of the present invention can undergo unlimited passages with substantially no differentiation. Examples 5 and 6 describe that the dissociated cell forms a cluster of undifferentiated cells after 7 days. Upon resuspension of the cells, new clusters form again 6 to 7 days later. The process was repeated in Example 6 a third time, thus showing that proliferation occurred at least 21 days *in vitro* with substantially no differentiation. The cells did not substantially differentiate until the methods of either claim 90 or 91 was followed. Accordingly, the method set forth in new claim 93, namely a method for proliferating a dissociated multipotent stem cell for at least 21 days *in vitro* with substantially no differentiation, is clearly distinguishable from the methods described by Cattaneo et al.

Claim 89 requires that the progenitor cells of the present invention be proliferated in suspension. The cells of Cattaneo I proliferate while adhered to a substrate (see Cattaneo's caption to Fig. 1), thus the element of proliferating the cells in suspension is not disclosed.

Claim 92 requires that the source of mammalian tissue, from which the multipotent stem cells are dissociated, be either from a

juvenile or adult mammal. Cattaneo et al. have only demonstrated minimal proliferation of unipotent neuronal precursor cells derived from embryonic tissue.

Thus because Cattaneo et al. do not disclose each and every element of the claimed invention, the reference is not anticipatory. Accordingly, it is believed that the rejection to claim 17 under § 102(a) is overcome.

Claim 17 also stands rejected under 35 U.S.C. § 102(b) as being anticipated by Morrison et al. The Examiner states that the cultured brain cell population of Morrison "is presumed to contain progenitor cells since the cells were able to differentiate." Progenitor cells are identified with antibodies that specifically bind to proteins found only in progenitor cells, such as nestin (see p. 29, lines 10-17 of specification). The majority of cells cultured by the methods of Morrison et al. exhibited neuronal morphology and were labeled by antibodies to a neuronal marker. The remainder of the cells were labeled by a marker for glial cells (see the paragraph bridging columns 2 and 3 on page 73 of Morrison). Because all of the cells cultured by the methods of Morrison et al. were identified by markers for differentiated cells, the Examiner's presumption that there must have been some progenitor cells present is unfounded. While there may or may not have been progenitor cells initially present in the neural tissue dissociated by Morrison et al., there is no teaching or suggestion whatsoever on how to culture and proliferate such cells if they in fact did originally exist in the culture. Thus a fundamental element of the claimed invention, namely the

proliferation of multipotent stem cells, is missing from the methods described by Morrison. Thus Morrison et al. does no more than teach that EGF promotes the survival and stimulates process outgrowth of neonatal rat CNS neurons *in vitro*. Process outgrowth is a characteristic of cell differentiation, and simply promoting survival does not mean proliferation.

Because Morrison et al. fail to disclose each and every element of the claimed invention the reference cannot be anticipatory. Therefore, the rejection to claim 17 under § 102(b) should be withdrawn.

Rejections under § 103

Applicants acknowledge the obligation under 37 C.F.R. § 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made. The inventions of each claim of the present application were commonly owned at the time made.

Claims 18 and 20 stand rejected under 35 U.S.C. § 103 as being unpatentable over Cattaneo et al. in view of Morrison et al. The present application discloses and claims the proliferation of multipotent stem cells in the presence of growth factors and the subsequent differentiation of the cells by either allowing the cells to adhere to a fixed substrate or by allowing the cells to form clusters of cells (neurospheres) without reinitiation of proliferation.

Cattaneo et al. disclose the limited proliferation of neuronal precursor cells, not multipotent stem cells capable of proliferation without limit. Cattaneo et al. fail to disclose or suggest how to proliferate cells that differentiate cell types other than neurons. Thus from this reference, one of ordinary skill in the art is provided no guidance on how to perform the methods of the claimed invention.

Cattaneo et al. also fail to disclose or suggest many of elements of the present invention that are set forth in the dependent claims. For example, the cells of Cattaneo et al. proliferate in response to NGF after they have been exposed to bFGF. The reference fails to suggest that EGF, amphiregulin, and TGFα induce the proliferation of progenitor cells, as set forth in claim 85. To the contrary, Cattaneo et al. state that the "combination of NGF or bFGF with other growth factors did not produce any effect on cell number" (p. 763, Col. 2), teaching that other growth factors do not have proliferative effects on neuronal progenitor cells. However, because the methods of the present invention are concerned with the proliferation of multipotent stem cells, and not neuronal precursor cells (which can only differentiate into neurons), the particular growth factors to which the cells are responsive may indeed be different.

The proliferation method of Cattaneo et al. requires that the cells be pre-exposed to serum (see legend to Figure 1), as was typically done in methods of culturing neural cells at the time of the present invention. The applicants have developed methods for

culturing, proliferating, and differentiating multipotent progenitor cells in completely defined conditions (i.e. without any preexposure to serum) as set forth in claim 87. Support for this new claim is found in the examples where the culture media used for dissociation, proliferation, and differentiation, is serum-free in each instance. The use of completely defined conditions for the cell culturing methods, especially methods where the cells are to be put to a therapeutic use, is highly desirable. Cattaneo et al. fail to teach or suggest how this can be accomplished.

Claim 89 sets forth the preferred method of proliferating multipotent stem cells, in suspension. While the multipotent stem cells can proliferate without differentiation while adhered to a substrate, the method of proliferation in suspension is preferred when large numbers of cells are to be generated (see specification p. 22, lines 20-23). Cattaneo et al. fail to suggest a method for the proliferation of neural cells (let alone multipotent stem cells) in suspension. The whole strategy of Cattaneo was based upon plated cells. At the time, plating cells was known to cause differentiation (see Weiss et al., PNAS 83:2238-2242 at 2240, (1986); cited by Examiner). Thus, Cattaneo et al. apparently overcame, to a limited extent, the tendency of neurons to differentiate when plated. Cattaneo et al., at best, demonstrate the proliferation for a period of 9 days *in vitro* ("DIV"). However, as set forth above, the cells of Cattaneo et al. were also differentiating during that period as illustrated by the increase in neurofilament staining cells. Thus, the approach of Cattaneo et al. is very different from that of the presently claimed invention.

Thus, for the above reasons, Cattaneo et al. fail to teach or suggest applicants' method of proliferating multipotent stem cells.

Morrison et al. teach that EGF promotes the survival and stimulates process outgrowth of neonatal rat CNS neurons in vitro, i.e. cell differentiation. The reference is wholly unconcerned with generating large numbers of multipotent stem cells (i.e. proliferation) and fails to teach or suggest how to do so. The Examiner notes that Morrison is "interpreted not [to] have stem cells or progenitor cells in the isolated population", but instead is cited to "disclose the use of EGF to induce brain cells to differentiate" (p. 6, lines 25-26 of Paper No. 8). Because Morrison is concerned only with differentiation and not with proliferation, and because with regards to the relationship between proliferation and differentiation the Examiner has stated that "it is known in the art that the two process[es] are distinct" (p 4. line 8-10), the Examiner must agree that a showing of the use of a growth factor to maintain survival and process outgrowth of differentiated cells would not suggest that the same growth factor could induce proliferation of stem cells. Indeed, it is known that differentiated cells have different cell-surface receptors than those of the stem cells from which they are derived. Thus, the fact that a differentiated neuronal cell, or even a neuronal precursor cell, is responsive to a given growth factor, does not necessarily mean that multipotent neural stem cells will be responsive to the same growth factor. Accordingly, Morrison et al. fail to teach or suggest the Applicant's use of growth factors to promote the proliferation of multipotent stem cells as set forth in claim 17.

As with Cattaneo et al., Morrison et al. also fail to disclose or suggest many of elements of the present invention that are set forth in the dependent claims. They do not culture the cells using defined conditions (see footnote 13) as set forth in claim 87. They do not use adult neural tissue, as set forth in claim 92.

If one of ordinary skill in the art were to combine the teachings of Cattaneo et al. with the teachings of Morrison et al., he would likely proliferate neuronal precursor cells in the presence of NGF and bFGF on a fixed substrate (according to the teachings of Cattaneo et al.), and then induce differentiation of the precursor cells into neurons by removing NGF and bFGF and adding EGF to the medium (according to the combined teachings of Cattaneo et al. and Morrison et al.). Such a combination does not result in nor suggest the claimed invention, namely proliferation of multipotent stem cells, and thus cannot render it obvious under § 103. For the above reasons, it is believed that the rejection to claims 18 and 20, as amended, is overcome.

Claims 17, 18 and 20 stand rejected under 35 U.S.C. § 103 as being unpatentable over Weiss et al. taken with Anchan et al. or Morrison et al. Weiss et al. show that neurons cultured on serum-coated substrates exhibit enhanced cell survival and neurite extension. As with Morrison et al., this reference is unconcerned with the proliferation of multipotent stem cells, and instead is concerned with already differentiated cells. Accordingly, this reference does not teach or suggest the claimed method.

Anchan et al. teach that EGF increases retinal germinal neuroepithelial cell proliferation when retinal cells are cultured as aggregates of more than 5 cells per cluster. There is no additional teaching whatsoever on the culture conditions used. Thus Anchan et al. is not an enabling disclosure, and cannot, even if combined with Weiss et al., teach or suggest the presently claimed methods., i.e. proliferation of a multipotent stem cell. Thus at the time the invention was made, the combined teachings of Anchan et al. and Weiss et al. would not have provided a reasonable expectation of success that multipotent stem cells obtained from mammalian neural tissue could be proliferated using the method set forth in claim 17, or differentiated using the methods of claim 87 or 88.

For the above reasons, it is believed that the rejection of claims 17, 18, and 20 is overcome.

Finally, it should be emphasized that by using the techniques of the present invention, one now has the capability of generating large numbers of multipotent stem cells from **adult brain tissue**. The proliferated cells are capable of differentiating into both neurons and glia. Before the Applicants' invention, it was commonly believed by neurobiologists that neurons could not be generated from adult tissue. It was believed that the brain gets all of its neurons during embryonic development and that none can be added in later life. Hence, all of the references cited by the Examiner pertain to research conducted on fetal tissue, the only tissue thought to be capable of generating neurons. Thus the Applicants' discovery shattered this theory. Accordingly, the

of its neurons during embryonic development and that none can be added in later life. Hence, all of the references cited by the Examiner pertain to research conducted on fetal tissue, the only tissue thought to be capable of generating neurons. Thus the Applicants' discovery shattered this theory. Accordingly, the methods of the present invention, and the use of adult brain tissue as a source for progenitor cells, as set forth in Claim 92, cannot be rendered obvious by the prior art.

In view of the foregoing, it is respectfully submitted that the present claims are in condition for allowance. Early notice of such allowance is solicited.

Respectfully submitted,

FLEHR, HOHBACH, TEST,
ALBRITTON & HERBERT

Jan P. Brunelle
Reg. No. 35,081

Four Embarcadero Center
Suite 3400
San Francisco, CA 94111-4187
Telephone: (415) 781-1989

Dated: 5 November 1993